The Importance of pH in the Regulation of Ruminal Acetate to Propionate Ratio and Methane Production In Vitro

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Introduction

Ruminal fermentation end products are dependent on diet, and acetate to propionate ratio is generally lower for cereal grains than for forages. When the acetate to propionate ratio decreases, there is a decline in CH_4 production, and energy retention by the cattle increases. Blaxter noted that the fraction of dietary energy that is converted to CH_4 declined significantly when the feed intake of the cattle increased, but the cause of this effect was not explained.

Cereal grain fermentation often causes a decrease in ruminal pH, and this effect is most dramatic when feed intake is high. Recent work has indicated that ruminal methanogens are sensitive to even modest decreases in pH. The question then arises, are decreases in acetate to propionate ratio that are dependent on cereal grain a product of low ruminal pH and the inability of methanogens to use reducing equivalents?

Materials and Methods

Ruminally fistulated cows were fed diets containing either 100% forage or 90% cereal grain and 10% forage (10 kg DM per day). Ruminal contents (1 L total) were squeezed through cheesecloth, and the pH was determined immediately. Concentrated ruminal bacteria were suspended in a medium containing salts, tricarballylate, branched chain volatile fatty acids, cysteine and transferred to tubes that contained 100 mg of hay or 50 mg of cracked corn and incubated for 48 h at 39°C. CH_4 and H_2 were measured with a gas chromatograph. Fermentation acids were analyzed with a high pressure liquid chromatograph.

Results

Cows that were fed 90% concentrate had lower ruminal pH values (6.22 vs. 6.86), higher VFA concentrations (85 vs. 68 mM), and lower acetate to propionate ratios (2.24 vs. 4.12) than did cows that were fed forage only. When mixed ruminal bacteria from cows that were fed 90% concentrate or 100% forage were incubated (48 h) with hay (10 g/L) or cracked corn (5 g/L) in a medium containing bicarbonate (38 mM) and tricarballylate (50 mM), final pH values were less than 0.3 units lower than the initial pH. At final pH values less than 5.7, hay fermentation was inhibited, the acetate to propionate ratio and CH_4 production declined more than two-fold, and the inoculum source was without effect. Small amounts of H_2 were detected at pH values less than 5.5. Total VFA production from cracked corn decreased when pH declined, but only if the inoculum was obtained from cows that were fed 90% concentrate. The acetate to propionate ratio of cracked corn incubations declined from 1.2 to 0.6 when final pH was decreased from 6.5 to 5.3, and CH_4, as a percentage of total VFA production, also decreased. At pH values less than 5.3, the acetate to propionate ratio of cracked corn increased more than four-fold, and large amounts of H_2 could be detected. Over the final pH range of 6.5 to 5.3, CH_4 production was highly correlated with acetate to propionate ratio, which was dependent on pH and substrate (CH_4 = 0.02 + 0.05 pH, r^2 = 0.80). Calculations based on the differences between pH 6.5 and 5.8 indicated that as much as 25% of the decrease in acetate to propionate ratio could be explained by the effect of pH alone.

Discussion

Inoculum source had no effect on total VFA from hay, and this result supports the assumption that ruminal cellulolytic bacteria cannot easily adapt to low pH. Our cracked corn fermentations were less sensitive to low pH than were hay fermentations, and the inocula differed in their pH sensitivity. Bacteria from cows that were fed 90% concentrate produced nearly as much VFA from corn at a final pH 5.2 as they did a pH of 6.5, but the bacteria from cows that were fed forage were strongly inhibited at pH values less than 5.5. This result supports the idea that some, but not all, starch-fermenting bacteria can adapt to low pH.

Low pH caused a marked decrease in the acetate to propionate ratio, and this decline was mirrored by a

reduction in CH_4 production. This result is consistent with the idea that propionate production and methanogenesis are competing and alternative mechanisms of reducing equivalent disposal, but this result alone provides little insight on the regulation. Was the increase in propionate driving the decrease in CH_4 or vice versa?

The pH had only a small impact on the acetate to propionate ratio and CH_4 production of cracked corn fermentations until the final pH was less than 5.5, and H_2 was not detected until the final pH was less than 5.5. Based on these results, it appears that many starch-fermenting bacteria prefer to produce propionate, and H_2 would not be available for methanogenesis.

Changes in the acetate to propionate ratio and CH_4 production were dependent on substrate and pH, but this simple correlation did not indicate which variable was more important (substrate or pH). By calculating the production of acetate and propionate from each feedstuff at pH 6.5 and 5.7, it was possible to develop a model to compare the effects of pH and substrate. When hay was replaced in stepwise fashion with corn, the acetate to propionate ratio always increased even if pH did not change, and this result indicated that substrate had a major impact on the fermentation end products .

Conclusions

The effect of pH was more subtle than substrate, but as much as 25% of the change in the acetate to propionate ratio could be explained by the change in pH. The relative effect of pH declined at high ratios of hay to corn, but cows that were fed hay do not usually have a low ruminal pH.